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OPERATION BIG BUZZ

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SUMMARY (S)

A field test was conducted in Georgia to demonstrate whether mosquitoes could be mass-produced and stored, loaded into munitions and disseminated from aircraft, and whether they would survive the airdrop and take blood meals from humans.

More than one million uninfected yellow-fever mosquitoes, Aedes aegypti, were produced and stored nearly two weeks. About one third of a million of these mosquitoes were concentrated in E14 munitions containing "aircomb waffles" and "loop tubes" and in 2½-ft rocket-shaped ground-release munitions, and dropped from aircraft without marked mortality. The remaining mosquitoes were used in munition loading and storage tests. Mosquitoes were airplane-released from munitions opening at altitudes from three hundred feet to ground level. They were dispersed by wind and their own flight and were collected as much as two thousand feet downwind from the target release site. The female mosquitoes were active in seeking blood meals and were attracted to and bit humans and guinea pigs.

This field test was a qualitative preliminary test to show feasibility of airplane delivery of mosquitoes for dissemination of BW agents. Since this test was successful, additional tests are planned using larger numbers of mosquitoes on adequate targets to permit quantitative evaluation of dispersal and survival, and to show feasibility of delivery of mosquitoes from a high-altitude jet bomber.

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OPERATION BIG BUZZ

I. PURPOSE (S)

The investigations were planned to obtain a qualitative determination of the survival and biting activity of mosquitoes confined in munitions and dropped from an airplane. These tests were of a preliminary nature, and were not designed to obtain either quantitative data on survival or any comprehensive assay of dispersal.

II. PRODUCTION OF TEST INSECTS (S)

The equipment and the general techniques for the mass-rearing of A aegypti, described in the Savannah CDC Contract Quarterly Report, April 1955, were utilized for the production of over one million adult mosquitoes. Shortcomings of the procedures were noted and constitute problems for future experimentation.

Two broods of mosquitoes, each consisting of 1,344,000 larvae, were set by placing 7,000 larvae in each of 192 trays, the latter each containing 7 liters of water. Five liters of the water was placed in each tray the day before the larvae were added. This water was treated with 0.1 gram of calcium hypochlorite which retarded the growth of micro-organisms within the pans and thus reduced scum formations. It was not detrimental to larval growth and development.

Using the Savannah CDC tray method for mass-rearing, 16.5 man-hours were required for the initial setting and feeding of a 192-tray batch of 1,344,000 larvae.

At the time of pupation, the larval trays were drained and larvae from 16 trays transferred into a single tray containing 10 liters of water treated with 0.1 gram of calcium hypochlorite. Twelve such trays were used, each fitted with an individual emergence cage. Larval feeding was continued in the emergence cages for 3 days, and fresh water was added to the emergence cage trays after the second day.

Adults were removed from the emergence cages daily. They were then anesthetized with carbon dioxide and transferred to 1-gallon ice cream cartons for storage. Approximately 1,500 adults were placed in each container and were fed a one-molar sucrose solution from a cotton pad placed on the nylon, broadcloth, or plastic screen top. Large cardboard packing cases, each containing 50 cartons, served as storage containers to facilitate handling of the cartons. The processes of removing, feeding, and storing each carton of adults required about 5 minutes.

The mosquitoes were held in a 335-cubic-foot walk-in refrigerator which was maintained at a mean temperature of 70°F and a mean relative humidity of 67 percent. The storage techniques resulted in excellent survival, and there was an estimated 90 percent survival of 10- to 12-day-old adults.

After storage, the mosquitoes were transferred from the gallon cartons into the munitions by means of vacuum suction. A household-type tank vacuum cleaner was used to aspirate the live adults from the cartons. They passed through the vacuum cleaner hose into the munition. A screen within the munition prevented their passage from it into the vacuum cleaner tank. The manometric vacuum was equivalent to the height of 1.5 to 2.0 inches of water. With this system it was possible to load mosquitoes in the munitions at the rate of 4 or 5 thousand per minute.

III. TEST SITES (S)

Two areas were utilized as test sites for the field studies. The initial tests were conducted at a U. S. Naval Auxiliary Air Station known as Harris Neck Field, located about 60 miles south of Savannah, Georgia. The 500-acre field is bordered by the South Newport River and is adjacent to salt marshes. All other tests were carried on at the Lynah Plantation, South Carolina, approximately 5 miles north of Savannah. This site was located in a cut-over ricefield now used as pasture. It covered approximately 500 acres. Each of the test areas was sparsely to densely covered by grasses 2 to 6 inches in height. Only a few trees or bushes were present at either test plot.

These test sites were selected in part because of the known absence of the test insect Aedes aegypti. Before the tests, careful studies were made by the field staff to show that none of these mosquitoes was indigenous to the area. These studies were duplicated and corroborated by several groups which conclusively showed the test insect to be absent from the area previous to the tests. All native species of mosquitoes in the area were sampled before and after the field tests. All mosquitoes collected from human observers and test animals following the airdrops were

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killed and taken to the laboratory for accurate determination including microscopic verification. The entomology staffs of both the USPHS and Camp Detrick concurred in the accuracy of the mosquito surveys.

Photographic coverage of the production of the test insects and of the test procedures was provided by Camp Detrick. Ground and air transportation and communication equipment were made available from the Technical Development Laboratories.

IV. TEST PROCEDURES (S)

Air and ground releases of mosquitoes from the munitions were carried out. The three munitions tested were the EL4 type with waffle-type (aircomb) inserts, the EL4 with tube inserts, and the 2½-ft rocket-shaped munition with an aircomb insert. The former two types provide an air release of the adult mosquitoes; the latter type provides a ground release of the mosquitoes. The munitions were dropped from an AT-6 airplane flying approximately 90 miles per hour at altitudes varying between 250 and 400 feet, but they opened and released the mosquitoes at lower elevations. Parachutes were used to slow the descent of one EL4 with waffle inserts and of all rocket-shaped munitions.

Mosquitoes were collected for assessment by three methods:

1. The Team Method

Each team was composed of two persons - one designated as the collector and the other as the bait. A team was assigned to work at a given radius from the target, and collected mosquitoes at intervals of 100 feet. The mosquitoes were collected from the human bait by the collector with the aid of an aspirator tube, an insect net, or both. Adult mosquitoes were chloroformed and placed in appropriately labeled boxes for later identification.

2. The Trap Method

Mosquito traps similar to those made from lard cans as described by Bellamy and Reeves in 1952 (Mosq. News, 12:256-258) were modified to permit the use of a live guinea pig as bait. They were operated from dusk on the day of the release until 4 or 5 hours after sunrise on the following day. The traps were then returned to the laboratory and the mosquitoes chloroformed and removed.

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3. The Spot Method

Direct observation and evaluation of the condition, activity, and biting rate of the mosquitoes were made as they reached the ground in waffle or disc inserts, or as they were released from the rocket aircomb inserts.

V. TEST RESULTS (S)

Date: May 24, 1955

Location: Harris Neck Field

Munition: E14 with waffle-type inserts

Release - 81,000 Aedes aegypti (27,000 per munition)

Mosquito age - 11 days

Loading time - 1300 - 1400

Storage time - until 1510

Storage conditions - 70°F and 67% RH

Drop time - 1615 - 1715

Wind: South - Southwest at 6 - 8 mph.

Mosquitoes were transferred from the laboratory to Saffold Field, approximately 3 miles from the loading site, and thence flown to the study area.

Trial	Altitude of Drop (feet)	Inserts Released		Remarks
		at Altitude (feet)*		
1	400	---	---	Dud
2	400	345	---	Dispersed
3	350	200	---	Dispersed
4	275	---	---	Dud
5	300	100	---	Dispersed
6	250	---	---	Dud

*Based on rate of fall estimated at 33 ft, first second; 66 ft, second second; and 100 feet/second thereafter.

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Munitions in trials 2, 3, and 5 expelled the waffle inserts in the dispersal pattern shown in Figure 1.

The munition in trial 4 broke open upon hitting the ground and was sprayed with a non-residual type of insecticide to prevent release of mosquitoes.

Fouling of the munition bridle caused the duds in trials 1 and 4. In trial 5 the bridle was removed to avoid possible fouling, small strips of masking tape being used to secure the munition's top in place until release. In trial 6, failure to remove the safety pin from the ejection mechanism resulted in a dud.

All the test insects were ejected from the waffle inserts in the air.

At approximately 1700, 1 female A aegypti was recovered in a collection from an observer near the target area. Drops 1 through 4 had been completed at this time. At 1730 the first team assessment began. Five males and one unfed female A aegypti were collected 1,000 feet from the target area. At 1915 (dusk) the second team assessment began. One engorged and one unfed female A aegypti were collected.

Five A aegypti (2 male and 3 female) were recovered from 4 of 16 mosquito traps. All recoveries of A aegypti are plotted in Figure 1.

Date: May 25, 1955

Location: Lynah Plantation, Savannah, Georgia

Munition: Rocket-shaped tube

Release - 75,000 Aedes aegypti (15,000 per munition)

Mosquito age - 11 - 12 days

Loading time - 1230 - 1400

Storage time - Until 1430

Storage conditions - 70°F and 67% RH

Drop time - 1520 - 1543

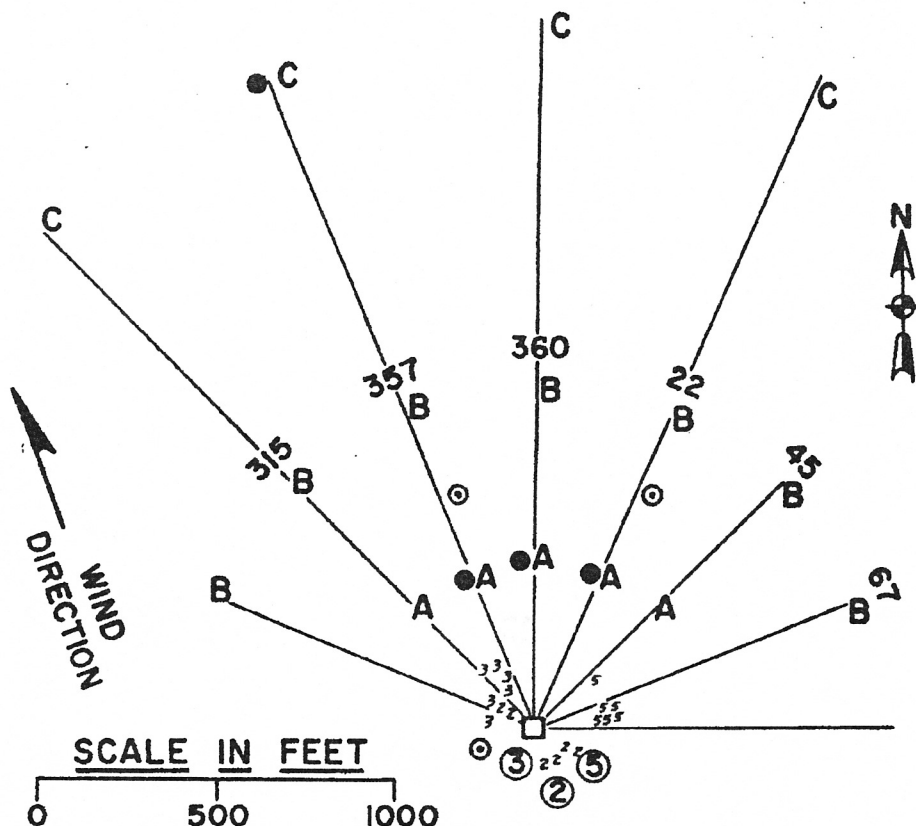
Wind: West at 7 mph.

Altitude of drops: 300 feet.

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Figure 1. Recovery pattern of *Aedes aegypti* and waffles from E14 munitions, Harris Neck, Ga., May 24, 1955.



LEGEND

- | | |
|-------------------------------------|--|
| □ - Target | A - Mosquito trap 500 feet from target |
| ② - Munition | B - Mosquito trap 1,000 feet from target |
| 2 - Inserts from specified munition | C - Mosquito trap 2,000 feet from target |
| ● - Positive trap collection | |
| ⊙ - Positive team collection | |

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The mosquitoes were transferred by car from the laboratory to Travis Field, approximately 15 miles distant, and then were flown to the test site.

Trial	Remarks
1	Chute failed, munition broken open on impact.
2	Chute fouled on airplane, munition not dropped on test site.
3	Chute opened and munition functioned properly.
4	As above.
5	As above.
6	Chute failed, munition broken open on impact.

The munition landing pattern in this test is shown in Figure 2.

In trials 1 and 6 some mosquitoes were found dead and others stunned in the opened rockets.

In trials 3, 4, and 5, large numbers of mosquitoes flew from the rockets as soon as the latter opened. Few dead mosquitoes were found in these trials. In the immediate vicinity of the rockets many A aegypti attacked and fed upon the field observers. Over 100 female A aegypti fed on the leg of one observer and all observers were bitten, even at a distance from the munitions.

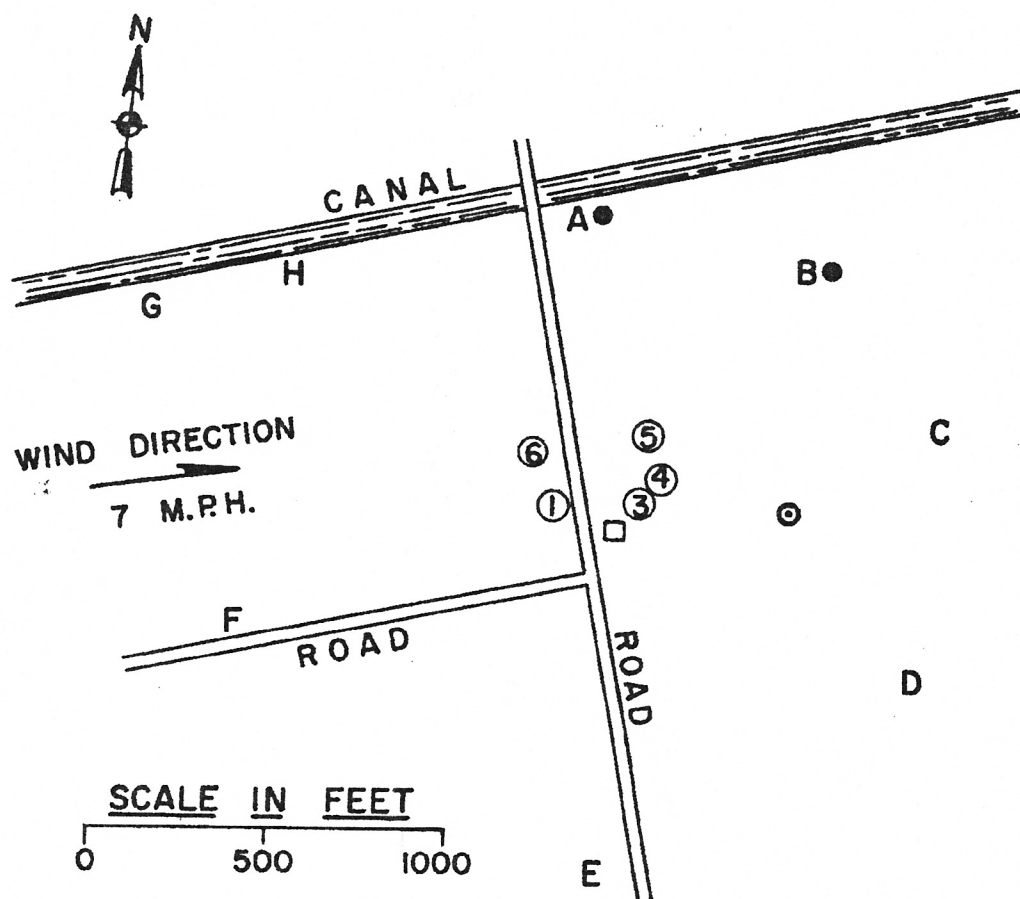
Four A aegypti (1 male and 3 females) were recovered from 2 of 8 mosquito traps on a circumference 1,000 feet from the target.

The morning following the release, a cursory assessment by 3 teams from 0900 until 1000 within 1,000 feet of the target area resulted in the recovery of 1 male and 1 female A aegypti. All recoveries of A aegypti are plotted in Figure 2.

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Figure 2. Landing pattern of rocket-shaped munitions and recovery sites of *Aedes aegypti*, Lynah Plantation, S. C., May 25 and 26, 1955.



LEGEND

- - Target
- ① - Munition
- A, B - Mosquito traps 1000 ft from target
- - Mosquito trap collection positive for *A. aegypti*
- ⊙ - Team collection positive for *A. aegypti*

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Date: May 26, 1955

Location: Lynah Plantation, Savannah, Georgia

Munitions: EL4 with waffle inserts and tube inserts

Release - 102,000 Aedes aegypti (51,000 per munition)

Mosquito age - 11 - 12 days

Loading time - 1350 - 1500

Drop time - 1605

Wind: Southwest < 10 mph.

Altitude of drops: 250 feet

The loaded munitions were transferred by car from the laboratory to Travis Field from which they were flown to the test site. An EL4 mock-up munition with waffle inserts was loaded with 51,000 mosquitoes and retained at the laboratory as a control to check mosquito mortality.

Trial	Inserts	Insert Release Altitude,* ft.
1	6 waffles	100/
2	76 tubes	100

*Estimated

The munitions were dropped over separate areas of the test site to avoid difficulties in making spot assessments of the mosquitoes from the two types of inserts.

In trial 1, a few mosquitoes were recovered from the waffle discs. None were obtained in sweep-net collections made in the vicinity of the inserts.

In trial 2, the tube inserts dispersed over an area approximately 30 x 150 feet. Many active mosquitoes were observed, particularly at one site where 15 tubes reached the ground attached to a cardboard filler from the munition. The female A. aegypti were active and successful in obtaining blood meals from the investigators.

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One tube contained 718 males and 723 females, all dead. Only a few living or dead mosquitoes were observed to remain in all other tubes for any length of time.

The mock-up munition at the laboratory was opened at approximately the same time as the field releases were being conducted. The mosquitoes readily escaped and attacked the observer. Mortality was estimated at 8 percent.

Date: May 27, 1955

Location: Lynah Plantation, Savannah, Georgia

Munitions: EL4 with waffle inserts and tube inserts

Release -- 60,000 Aedes aegypti (30,000 per munition)

Mosquito age -- 3 - 4 days

Loading time -- 1300 - 1400

Drop time -- 1620

Wind: Southwest 7 mph.

After loading, the munitions were transported by car from the laboratory to Travis Field. The flight to the study site was delayed approximately 1.5 hours by localized thundershowers.

Trial	Altitude of Drop (feet)	Inserts
1	300	6 waffles
2	250	65 tubes

The munitions were dropped into separate areas of the test site to avoid difficulties in making a spot assessment of the mosquito from the two types of inserts.

In trial 1, a parachute attached to the munition opened at about 200 feet elevation. Spot assessment revealed biting A aegypti in the immediate area of the inserts which fell in a circular area 20 feet in diameter. Sweep-net collections from 5 to 500 feet on the lee side of this area were negative.

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In trial 2, the tube inserts landed in an area of 30 x 50 feet. A spot assessment showed active, biting mosquitoes near the tubes. Some tubes were recovered containing a few live adults; none contained dead mosquitoes.

VI. DISCUSSION (S)

OPERATION BIG BUZZ was designed to answer the questions:

1. Can large numbers of mosquitoes survive concentration in munitions in sufficient numbers to make air drops a practical procedure?
2. Will mosquitoes survive air and ground release from the munitions?
3. Will mosquitoes actively disperse and seek blood meals?

Field and laboratory tests proved it possible to hold the mosquitoes in the munitions for the time necessary for transport to the aircraft and to the test site. Large numbers of mosquitoes were successfully loaded into both the EL4 and the rocket-type munitions. Given advance knowledge of any standard munition, a simple but efficient apparatus could be developed to expedite loading the mosquitoes into the munition. A system whereby adults could be transferred directly from emergence cages to munitions would be desirable.

Mosquitoes survived the air releases from the EL4 munitions and the ground releases from the rockets. The smallest recoveries of living mosquitoes were from EL4 munitions with waffle inserts. Several tube inserts from EL4 munitions were found to contain living mosquitoes, and small concentrations of adults were observed in the vicinity of the inserts. In contrast, the rocket-type munitions giving a ground release provided the most spectacular concentrations of live adults. With each type of munition tested, A aegypti were recovered at varying distances from the points of release and sought blood meals and bit humans.

On the basis of recovery of adults, the ground-release rocket-shaped munition was the most effective type of munition. This device, however, limits dispersion of the mosquitoes to the natural capabilities of the species, which may not be great. In contrast, the EL4 munition with waffle inserts provides a wider dispersal of the mosquitoes by virtue of being released above ground. In this type of release the mosquito dispersion is most influenced by wind and by the altitude at which the mosquitoes are ejected. Observers believed that the rate of fall of the munition should be reduced before the ejection of the mosquitoes in an air release.

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The EL4 with the tube inserts provided a combination air and ground release. Those mosquitoes resting between the tube inserts in the munitions are released in the air when the tubes are ejected. Many adults leave the tubes during their descent, but a few mosquitoes remain within the tube inserts until they reach the ground and then disperse.

In reviewing the results of these tests consideration must be given to the relatively small populations of mosquitoes released in relation to the vast areas involved, and to the inadequacies of the sampling techniques. These factors, particularly the first, make it remarkable that so many mosquitoes were recovered.

Any quantitative assessment of survival and dispersal would require the release of at least a million mosquitoes in a suitable test site. It would necessitate intensive mosquito collecting over an extended period of time. With increased space, equipment, and manpower, a single brood production of one or several million mosquitoes of nearly uniform age could be produced for such tests.

The development of a reliable munition to provide the desired type of mosquito release was shown to be feasible. The human and mechanical errors which resulted in the failure of several munitions are of relatively little consequence. That the functioning ones produced the desired mosquito release and activity is significant.

The three assessment methods utilized to obtain the qualitative data on survival and biting activity were satisfactory. The team and the trap methods are potential tools for use in future studies designed to obtain quantitative data upon mosquito survival and dispersion.

The number of A aegypti that may be laboratory-reared is theoretically unlimited; practical limitations include available space and manpower. The man-hours per unit of production undoubtedly could be further reduced by development and installation of relatively simple water storage and conduit equipment.

The currently available facilities and techniques were adequate for production of over one million mosquitoes; however, two successive broods were needed to supply this number. About 465,000 of the adults produced were used in the tests reported and in preliminary experiments. In future tests the use of adults from a single brood (about 500,000 under present conditions) would provide test insects of uniform age and would avoid unnecessary handling and storage. Continuing investigations on biological requirements resulting in the development of improved methods for mass-production may provide the means for producing more adults per brood.

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